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BIANCHI'S CAVE SALAMANDER,  
*SPELEOMANTES AMBROSII BIANCHII* N. SSP.

(AMPHIBIA, CAUDATA, PLETHODONTIDAE)

## INTRODUCTION

NASCETTI *et alii* (1996) and FORTI *et alii* (1998) showed that *Speleomantes ambrosii* (Lanza, 1955) consists of two well differentiated forms ( $D_{\text{Nei}}=0.18$ ) living respectively W and E of the Magra River (NW Italy). The first one, inhabiting the province of La Spezia (Liguria), *terra typica* of the species, corresponds to the nominate taxon *Speleomantes ambrosii ambrosii*, while the second one, typical of the province of Massa-Carrara (Tuscany), has to be described as *Speleomantes ambrosii bianchii* n. ssp.

## MATERIALS AND METHODS

Genetic variation was investigated in 174 specimens taken from 8 localities covering the whole range of the species. Three localities are inhabited by *S. ambrosii bianchii* and the other five by *S. ambrosii ambrosii* (FORTI *et alii* 1998). An overall number of 33 enzyme

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Table 1 - The enzymes studied in *S. a. ambrosii* and *S. a. bianchii*, with the encoding loci, the electrophoretic conditions and the staining references. Migration: + = anodal, - = cathodal. Buffer system: 1) Tris-Versene-Borate (BREWER & SING 1970); 2) Tris-Versene-Maleate (BREWER & SING 1970); 3) Phosphate-Citrate (HARRIS 1966); 4) Discontinuous Tris-Citrate (POULIK 1957); 5) Continuous Tris-Citrate (SELANDER *et alii* 1971). Staining references: a) BREWER & SING (1970); b) SHAW & PRASAD (1970); c) SELANDER *et alii* (1971); d) AYALA *et alii* (1972); e) HARRIS & HOPKINSON (1976); f) RICHARDSON *et alii* (1986).

| Enzyme                                                            | E.C.<br>number | Encoding<br>loci | Migration | Buffer | V/cm | Running<br>time (hr) | Staining<br>ref. |
|-------------------------------------------------------------------|----------------|------------------|-----------|--------|------|----------------------|------------------|
| $\alpha$ -Glycerophosphate dehydrogenase<br>Lactate dehydrogenase | 1.1.1.8        | $\alpha$ -Gpdh   | +         | 3      | 8    | 6                    | d                |
|                                                                   | 1.1.1.27       | Ldh-1            | +         | 4      | 7    | 5                    | a                |
| Hydroxybutyrate dehydrogenase                                     | 1.1.1.30       | Ldh-2            | -         | 2      | 7    | 6                    | b                |
|                                                                   | 1.1.1.37       | Hbdh             | +         | 3      | 8    | 6                    | b                |
| Malate dehydrogenase                                              |                | Mdh-1            | +         | 5      | 7    | 6                    | d                |
|                                                                   |                | Mdh-2            | +         | 5      | 7    | 5                    | b                |
| Malate dehydrogenase (NADP+)                                      | 1.1.1.40       | Mdhp-1           | +         | 3      | 8    | 6                    | b                |
|                                                                   |                | Mdhp-2           | +         | 3      | 8    | 6                    | b                |
| Isocitrate dehydrogenase                                          | 1.1.1.42       | Idh-1            | +         | 5      | 7    | 6                    | d                |
|                                                                   |                | Idh-2            | +         | 5      | 7    | 5                    | b                |
| 6-Phosphogluconate dehydrogenase                                  | 1.1.1.44       | 6-Pgdh           | +         | 3      | 8    | 6                    | b                |
| Glyceraldehyde-3-phosphate<br>dehydrogenase                       | 1.2.1.12       | Gapdh            | +         | 5      | 7    | 6                    | d                |
|                                                                   | 1.6.99.3       | NADH-dh          | +         | 2      | 8    | 8                    | a                |
| NADH dehydrogenase                                                | 1.15.1.1       | Sod-1            | +         | 1      | 7    | 7                    | c                |
| Superoxide dismutase                                              |                | Sod-2            | +         | 1      | 8    | 6                    | e                |
| Nucleoside phosphorilase                                          | 2.4.2.1        | Np               | +         | 3      | 8    | 5                    | c                |
|                                                                   | 2.6.1.1        | Aat-1            | +         | 5      | 7    | 5                    | b                |
| Aspartate-aminotransferase                                        |                | Aat-2            | -         | 5      | 7    | 5                    | d                |
|                                                                   |                | Ck               | +         | 5      | 7    | 5                    | d                |
| Creatine kinase                                                   | 2.7.3.2        | Adk              | +         | 4      | 8    | 5                    | d                |
| Adenylate kinase                                                  | 2.7.4.3        | Est-4            | +         | 5      | 7    | 4                    | f                |
| Esterase                                                          | 3.1.1.1        | Pep-C            | +         | 5      | 7    | 4                    | f                |
| Peptidase (Leu-Ala)                                               | 3.4.11.        | Pep-D            | +         | 4      | 7    | 4                    | d                |
| Peptidase (Phe-Pro)                                               | 3.4.11.        | Lap              | +         | 5      | 7    | 5                    | e                |
| Leucine aminopeptidase                                            | 3.4.11.        | Ada-1            | +         | 1      | 7    | 4                    | e                |
| Adenosine deaminase                                               | 3.5.4.4        | Ada-2            | +         | 1      | 7    | 4                    | e                |
| Carbonic anhydrase                                                | 4.2.1.1        | Ca-2             | +         | 1      | 7    | 4                    | e                |
| Fumarase                                                          |                | Ca-3             | +         | 1      | 7    | 6                    | b                |
|                                                                   | 4.2.1.2        | Fum              | +         | 1      | 7    | 6                    | e                |
| Mannose phosphate isomerase                                       | 5.3.1.8        | Mpi              | +         | 3      | 8    | 6                    | c                |
| Glucose phosphate isomerase                                       | 5.3.1.9        | Gpi              | -         | 3      | 8    | 6                    | c                |
| Phosphoglucomutase                                                | 5.4.2.2        | Pgm-1            | +         | 3      | 8    | 6                    | c                |
|                                                                   |                | Pgm-2            | +         |        |      |                      |                  |



The morphological data reported in the present paper, unless specified otherwise, refer only to adults, to avoid the variation due to allometric growth or to other types of ontogenetic changes. Measurements and counting methods were as follows:

total length (tl) (only in the specimens with tail unregenerated or seemingly so);

body length (bl)= snout tip-anterior end of the vent;

axilla-groin (ag);

tail length (tal)= total length minus body length;

head length (hl)= snout tip-oral angle (marked by a thin needle);

head width (hw)= between the oral angles, each marked by a thin needle;

head depth (hd)= distance between the tangent of the lower jaw and the highest point of the head;

lower lip-gular fold (lg)= distance between the midpoint of the lower lip and the midpoint of the gular fold;

snout length (sl)= from the midpoint of the upper lip border to the anterior eyelid commissure;

internarial distance (id)= shortest distance between the median margins of the narial openings;

orbitonarial distance (od)= between the posterior border of the narial openings and the anterior eyelid commissure;

intercanthal distance (icd)= between each anterior eyelid commissure;

interorbital distance (iod)= the shortest distance between the medial margin of the upper eyelids;

eye diameter (ed)= distance between anterior and posterior eyelid commissures;

mental gland width (mgw)= its greatest width;

mental gland length (mgl)= from the anterior midpoint, just behind the mandibular symphysis, to the midpoint of the posterior border (when the latter is concave, to the midpoint of the tangent touching its posteriormost points);

snout-epibranchial tip (se)= distance between the tip of the snout and the posterior end of the epibranchial sheath with the tongue at rest position (the end of the sheath exposed by dissection);

foreleg length (fl)= from its anterior attachment to the tip of the 3rd finger;



hind leg length (hil)= from the groin to the tip of the longest toe;  
 foot length (fol)= from the inner border of the foot to the tip of the  
 4th toe, the latter at 90° against the inner margin of the foot (cf.  
 LANZA *et alii* 1995, fig. 1);

teeth number (approximate)= tooth counts are based on the visible  
 teeth, i.e. on the functional, ankylosed teeth, as well as on those  
 not yet functional but almost piercing the mucous membrane  
 and about to replace the lost ones (on one side; of course teeth  
 lacking due to traumatism were also counted as present) (pm=  
 premaxillar teeth; av= anterior vomerine teeth; m= maxillary  
 teeth; ma= mandibular teeth);

trunk vertebrae (tv)= number of the trunk vertebrae (i.e. of the pre-  
 sacral vertebrae, atlas excluded), usually counted on radiographs,  
 sometimes in skeletonized and double-stained animals;

postsacral vertebrae (pv)= number of the sacro-caudal and caudal  
 vertebrae, comprising the smallest apical, radiographically  
 detectable elements.



Fig. 1 - *Speleomantes ambrosii ambrosii*; ♀ 23323 MZUF (formerly 294 NHCL = New Herpetological Collection Lanza); Grotta dei Pitalli (= Grotta dell'Ospizio), cadastral number 1214 Li/SP, 100 m a.s.l. [44°07'15"N-02°39'21"W (Rome)= 09°47'47"E Greenwich]; locality Chiappa; province and municipality of La Spezia; leg. S. Vanni & P. Magrini, 17.IX.1989 (photo Stefano Vanni).



The following ratios were taken: 1= total length: tail length; 2= body length: axilla-groin; 3= body length: foreleg length; 4= body length: hind leg length; 5= hind leg length: foreleg length; 6= body length: head length; 7= body length: head width; 8= body length: lower lip-gular fold; 9= body length: foot length; 10= body length: mental gland width; 11= body length: mental gland length; 12= mental gland width: mental gland length; 13= body length: snout-epibranchial tip; 14= head length: head width; 15= head length: head depth; 16= head length: snout length; 17= head length: internarial distance; 18= head length: orbitonarial distance; 19= head length: intercanthal distance; 20= head length: interorbital distance; 21= head length: eye diameter; 22= internarial distance: interorbital distance.

Since the genome of most populations of *S. a. bianchii* is more or less introgressed by genes of *Speleomantes italicus* (Dunn, 1923) (see especially RUGGI *et alii* 2005), we chose to describe morphologically the new taxon only on the basis of the westernmost “pure” populations of the province of Massa-Carrara, i.e. those from Pùlica-Marciaso’s zone (see “Results” for the material’s list).

Morphology and pattern of *S. ambrosii bianchii* from Pùlica-Marciaso’s area have been compared with those of the following 30 parapatric specimens of *S. ambrosii ambrosii*, all collected in the type locality and environs (eastern end of the province of La Spezia; Liguria); all the specimens quoted in the present paper belong to the collections of the Museo Zoologico dell’Università di Firenze (MZUF) and numbers are referred to the catalogue of MZUF, but two paratypes of *S. ambrosii bianchii* which are deposited in the collection of the Museo Civico di Storia Naturale “G. Doria” of Genoa (MSNG); c.n.= cadastral number of the caves:

♀♀ 13609, Manarola, along the path to Corniglia (Cinqueterre), leg. S. Taiti, 28.XII.1980;

3 ♂♂ 1723, 1724 and 1725, ♀ 1726 (paratypes of *Hydromantes italicus ambrosii*), cave Sprugola di Monte Tenero (= Caverna di Monte Tenero n. 1), c.n. 236 Li/SP, 44°07’42”N-02°40’28”W(Rome), 250 m, leg. A. C. Ambrosi, 11.VII.1953;

3 ♀♀ 1716, 1717 and 1718 (paratypes), cave Caverna della Taggia (= Antro del Pantano; Sprugola della Taggia), c.n. 79 Li/SP, 44°06’03”N-02°39’47”W (Rome), 210 m, locality Colle Matellina, near Pegazzano, leg. A. C. Ambrosi, IV.1953;



Table 2 - Statistical analysis of 22 morphological ratios in *Speleomantes ambrosii bianchii* n. ssp. from Pùlica and *S. ambrosii ambrosii* from La Spezia  
t=Student's test. Ratios are defined in the section Materials and Methods.

| Locality | Sex | n  | Ratio | Range     | Mean       | cl (99%)  | t               | df | P     |
|----------|-----|----|-------|-----------|------------|-----------|-----------------|----|-------|
| Spezia   | ♂♂  | 10 | 1     | 2.20-2.41 | 2.32±0.074 | 2.25-2.39 |                 |    |       |
|          |     |    |       |           |            |           | 3.32            | 24 | <0.01 |
| Pùlica   | ♂♂  | 16 | 1     | 2.15-2.33 | 2.24±0.057 | 2.19-2.28 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♀♀  | 15 | 1     | 2.14-2.42 | 2.31±0.067 | 2.26-2.37 |                 |    |       |
|          |     |    |       |           |            |           | 2.92            | 19 | <0.01 |
| Pùlica   | ♀♀  | 6  | 1     | 2.11-2.31 | 2.22±0.073 | 2.09-2.34 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♂♂  | 12 | 2     | 1.86-2.07 | 1.98±0.057 | 1.93-2.03 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♂♂  | 16 | 2     | 1.88-2.09 | 1.99±0.065 | 1.94-2.04 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♀♀  | 18 | 2     | 1.78-2.00 | 1.90±0.056 | 1.86-1.93 |                 |    |       |
|          |     |    |       |           |            |           | 3.40            | 23 | <0.01 |
| Pùlica   | ♀♀  | 7  | 2     | 1.85-2.13 | 1.99±0.091 | 1.87-2.12 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♂♂  | 12 | 3     | 3.08-3.43 | 3.26±0.103 | 3.17-3.35 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♂♂  | 16 | 3     | 2.91-3.40 | 3.20±0.121 | 3.11-3.29 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♀♀  | 18 | 3     | 2.88-3.54 | 3.31±0.160 | 3.20-3.42 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♀♀  | 7  | 3     | 3.21-3.45 | 3.31±0.086 | 3.19-3.43 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♂♂  | 12 | 4     | 2.95-3.36 | 3.12±0.118 | 3.01-3.23 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♂♂  | 16 | 4     | 2.86-3.38 | 3.11±0.160 | 3.00-3.23 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♀♀  | 18 | 4     | 2.82-3.41 | 3.12±0.167 | 3.01-3.24 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♀♀  | 7  | 4     | 3.00-3.24 | 3.16±7.900 | 3.04-3.27 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♂♂  | 12 | 5     | 0.97-1.10 | 1.05±0.042 | 1.01-1.08 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♂♂  | 16 | 5     | 0.93-1.1  | 1.03±0.044 | 0.99-1.06 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♀♀  | 18 | 5     | 1.00-1.12 | 1.06±0.038 | 1.03-1.08 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♀♀  | 7  | 5     | 1.02-1.07 | 1.05±0.022 | 1.02-1.08 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♂♂  | 9  | 6     | 5.05-5.39 | 5.27±0.107 | 5.15-5.39 |                 |    |       |
|          |     |    |       |           |            |           | 4.48            | 23 | <0.01 |
| Pùlica   | ♂♂  | 16 | 6     | 5.31-5.90 | 5.54±0.167 | 5.42-5.67 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♀♀  | 10 | 6     | 5.22-5.82 | 5.50±0.228 | 5.27-5.74 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♀♀  | 7  | 6     | 5.33-5.90 | 5.69±0.192 | 5.42-5.96 |                 |    |       |
|          |     |    |       |           |            |           |                 |    |       |
| Spezia   | ♂♂  | 9  | 7     | 5.68-6.19 | 5.92±0.208 | 5.69-6.15 |                 |    |       |
|          |     |    |       |           |            |           | no significance |    |       |
| Pùlica   | ♂♂  | 16 | 7     | 5.35-6.49 | 6.11±0.314 | 5.88-6.34 |                 |    |       |



Table 2 - (continued) Statistical analysis of 22 morphological ratios in *Speleomantes ambrosii bianchii* n. ssp. from Pùlica and *S. ambrosii ambrosii* from La Spezia. Ratios are defined in the section Materials and Methods.

|        |    |    |    |             |             |             |                 |    |       |
|--------|----|----|----|-------------|-------------|-------------|-----------------|----|-------|
| Spezia | ♀♀ | 10 | 7  | 5.69-6.38   | 5.96±0.198  | 5.76-6.17   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♀♀ | 7  | 7  | 5.70-6.10   | 5.88±0.126  | 5.71-6.06   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♂ | 12 | 8  | 3.94-4.54   | 4.22±0.217  | 4.02-4.41   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♂♂ | 16 | 8  | 3.22-4.55   | 4.18±0.286  | 3.97-4.39   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♀♀ | 13 | 8  | 3.88-4.61   | 4.23±0.204  | 4.06-4.40   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♀♀ | 7  | 8  | 4.11-4.35   | 4.26±0.087  | 4.13-4.38   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♂ | 12 | 9  | 7.20-8.21   | 7.62±0.290  | 7.36-7.88   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♂♂ | 16 | 9  | 7.05-8.16   | 7.50±0.294  | 7.29-7.72   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♀♀ | 18 | 9  | 7.16-8.51   | 7.80±0.403  | 7.52-8.07   | 2.13            | 23 | <0.05 |
| Pùlica | ♀♀ | 7  | 9  | 7.06-8.26   | 7.40±0.419  | 6.82-7.99   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♂ | 12 | 10 | 13.17-16.87 | 14.81±1.112 | 13.81-15.81 |                 |    |       |
|        |    |    |    |             |             |             | 5.30            | 26 | <0.01 |
| Pùlica | ♂♂ | 16 | 10 | 11.22-14.88 | 12.62±1.055 | 12.06-13.40 |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♂ | 12 | 11 | 18.00-22.92 | 21.62±1.518 | 20.26-22.90 |                 |    |       |
|        |    |    |    |             |             |             | 9.23            | 26 | <0.01 |
| Pùlica | ♂♂ | 16 | 11 | 15.14-18.71 | 17.47±0.847 | 16.84-18.09 |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♂ | 12 | 12 | 1.02-1.68   | 1.42±0.163  | 1.28-1.57   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♂♂ | 16 | 12 | 1.17-1.58   | 1.39±0.106  | 1.31-1.47   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♀ | 20 | 13 | 1.78-2.15   | 1.97±0.130  | 1.88-2.05   |                 |    |       |
|        |    |    |    |             |             |             | 3.45            | 38 | <0.01 |
| Pùlica | ♂♀ | 20 | 13 | 1.88-2.29   | 2.10±0.117  | 2.03-2.17   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♂ | 9  | 14 | 1.06-1.18   | 1.12±0.039  | 1.08-1.17   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♂♂ | 16 | 14 | 1.03-1.17   | 1.11±0.042  | 1.08-1.14   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♀♀ | 10 | 14 | 1.03-1.15   | 1.08±0.037  | 1.05-1.12   |                 |    |       |
|        |    |    |    |             |             |             | 4.17            | 21 | <0.01 |
| Pùlica | ♀♀ | 7  | 14 | 1.00-1.08   | 1.04±0.030  | 0.99-1.08   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♂♂ | 9  | 15 | 1.89-2.51   | 2.25±0.194  | 2.03-2.47   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♂♂ | 16 | 15 | 1.70-2.52   | 2.10±0.232  | 1.93-2.27   |                 |    |       |
|        |    |    |    |             |             |             |                 |    |       |
| Spezia | ♀♀ | 10 | 15 | 2.00-2.28   | 2.16±0.083  | 2.08-2.25   |                 |    |       |
|        |    |    |    |             |             |             | no significance |    |       |
| Pùlica | ♀♀ | 7  | 15 | 1.89-2.35   | 2.13±0.157  | 1.91-2.35   |                 |    |       |



Table 2 - (continued) Statistical analysis of 22 morphological ratios in *Speleomantes ambrosii bianchii* n. ssp. from Pùlica and *S. ambrosii ambrosii* from La Spezia. Ratios are defined in the section Materials and Methods.

|        |    |    |    |           |            |           |  |                 |    |  |       |
|--------|----|----|----|-----------|------------|-----------|--|-----------------|----|--|-------|
| Spezia | ♂♂ | 9  | 16 | 2.12-2.48 | 2.30±0.126 | 2.16-2.44 |  | 2.14            | 23 |  | <0.05 |
| Pùlica | ♂♂ | 16 | 16 | 2.00-2.32 | 2.21±0.081 | 2.15-2.27 |  |                 |    |  |       |
| Spezia | ♀♀ | 10 | 16 | 2.19-2.44 | 2.29±0.083 | 2.21-2.38 |  |                 |    |  |       |
| Pùlica | ♀♀ | 7  | 16 | 2.04-2.32 | 2.15±0.097 | 2.01-2.28 |  | 3.27            | 15 |  | <0.01 |
| Spezia | ♂♂ | 9  | 17 | 2.89-3.50 | 3.09±0.185 | 2.89-3.30 |  |                 |    |  |       |
| Pùlica | ♂♂ | 16 | 17 | 2.60-3.10 | 2.81±0.136 | 2.71-2.90 |  | 4.50            | 23 |  | <0.01 |
| Spezia | ♀♀ | 10 | 17 | 2.91-3.41 | 3.16±0.149 | 3.01-3.31 |  |                 |    |  |       |
| Pùlica | ♀♀ | 7  | 17 | 2.78-3.29 | 2.98±0.179 | 2.73-3.23 |  | 2.31            | 15 |  | <0.05 |
| Spezia | ♂♂ | 9  | 18 | 3.43-3.92 | 3.70±0.168 | 3.50-3.88 |  |                 |    |  |       |
| Pùlica | ♂♂ | 16 | 18 | 2.54-3.48 | 3.22±0.225 | 3.05-3.38 |  | 5.50            | 23 |  | <0.01 |
| Spezia | ♀♀ | 10 | 18 | 3.30-3.89 | 3.65±0.220 | 3.43-3.88 |  |                 |    |  |       |
| Pùlica | ♀♀ | 7  | 18 | 2.97-3.57 | 3.29±0.222 | 2.98-3.60 |  | 3.38            | 15 |  | <0.01 |
| Spezia | ♂♂ | 9  | 19 | 2.12-2.41 | 2.25±0.110 | 2.13-2.37 |  |                 |    |  |       |
| Pùlica | ♂♂ | 16 | 19 | 1.94-2.30 | 2.10±9.999 | 2.03-2.17 |  | 3.69            | 23 |  | <0.01 |
| Spezia | ♀♀ | 10 | 19 | 2.06-2.43 | 2.18±0.105 | 2.07-2.29 |  |                 |    |  |       |
| Pùlica | ♀♀ | 7  | 19 | 1.83-2.27 | 2.01±0.137 | 1.82-2.20 |  | 2.90            | 15 |  | <0.02 |
| Spezia | ♂♂ | 9  | 20 | 3.25-3.92 | 3.60±0.196 | 3.39-3.83 |  |                 |    |  |       |
| Pùlica | ♂♂ | 16 | 20 | 3.00-4.00 | 3.49±0.321 | 3.25-3.72 |  | no significance |    |  |       |
| Spezia | ♀♀ | 10 | 20 | 3.25-4.04 | 3.61±0.259 | 3.34-3.87 |  |                 |    |  |       |
| Pùlica | ♀♀ | 7  | 20 | 2.81-3.57 | 3.18±0.274 | 2.80-3.57 |  | 3.26            | 15 |  | <0.01 |
| Spezia | ♂♂ | 9  | 21 | 2.61-2.97 | 2.83±0.122 | 2.70-2.97 |  |                 |    |  |       |
| Pùlica | ♂♂ | 16 | 21 | 2.82-3.25 | 2.97±0.123 | 2.88-3.06 |  | 2.71            | 23 |  | <0.02 |
| Spezia | ♀♀ | 10 | 21 | 2.64-3.00 | 2.84±0.105 | 2.73-2.95 |  |                 |    |  |       |
| Pùlica | ♀♀ | 7  | 21 | 2.68-2.94 | 2.81±0.084 | 2.69-2.93 |  | no significance |    |  |       |
| Spezia | ♂♂ | 12 | 22 | 1.03-1.35 | 1.16±0.100 | 1.07-1.25 |  |                 |    |  |       |
| Pùlica | ♂♂ | 16 | 22 | 1.04-1.44 | 1.24±0.124 | 1.14-1.33 |  | no significance |    |  |       |
| Spezia | ♀♀ | 14 | 22 | 0.96-1.30 | 1.14±0.091 | 1.06-1.21 |  |                 |    |  |       |
| Pùlica | ♀♀ | 7  | 22 | 0.93-1.28 | 1.07±0.136 |           |  | no significance |    |  |       |



4 ♂♂ 15943, 15945, 15946 and 15947, 4 ♀♀ 15942, 15944, 15948 and 15949, outdoors, close to the above-mentioned cave, 150-220 m, leg. B. Lanza, P. Finotello & P. Malenotti, 14.II.1982; 4 ♂♂ 19674, 19675, 19676 and 19677, ♀ 19679, *ibidem*, leg. B. Lanza & G. Tosini, 18.XII.1989;

♂ 15965, ♀ 15964, La Spezia (in the town, under stones beside the road to Bocca Lupara), ca. 80 m, leg. S. Vanni & P. Magrini, 2.IV.1985;

♀ 1727 (holotype), and 3 ♀♀ 1728, 1729 and 1730 (paratypes), cave Sprugola della Cornese (= Sprugola la Canese), c.n. 186 Li/SP, 44°05'15"N-02°39'14"W (Rome), near Fabiano, 240 m, leg. A. C. Ambrosi, 23.IX.1952;

♂ 1584, Ceppo, ca. 500 m SW of Fabiano (SP), leg. G. Pini, 31.X.1954;

3 ♀♀ 1623, 1624 and 1625 (paratypes), cave La Paladina, c.n. 1208 Li/SP, 44°05'02"5N-02°38'21"W (Rome), Capoacca Valley, Coregna on the Mt Castellano, 240 m, leg. A. C. Ambrosi, III and IV.1953.

## RESULTS

### *Speleomantes ambrosii bianchii* n. ssp. (figs 2-6)

Holotype: Adult ♂ 26107 (formerly 4452 New Herpetological Collection Lanza = NHCL); cave Tana (or Buca) della Bastiòla, c.n. 481 T/MS, 44°08'08"N-02°24'08"W (Rome), on the N slope of Mt Bastione (698 m) and on the left side of the Rio Grande, about 1.2 km SSE of Pùlica (church) and 2.3 km E of Fosdinovo, 360 m (municipality of Fosdinovo; locality Rio Grande); leg. B. Lanza & S. Angelotti, 11.X.1997.

Paratypes - 65 specimens as follows:

- same locality as holotype, ♂ 1884, 2 ♀♀ 1883 and 1885, leg. T. Bianchi & B. Lanza, 18.X.1942; ♀ 6611, leg. B. Lanza, 28.X.1945; 9 juv. 1874, 1875, 1876, 1877, 1878, 1879, 1880, 1881, 1882, leg. E. Bianchi, 10.VI.1952; juv. 5225, ♂ 5223, ♀ 5224, leg. B., L., & M. Lanza, 19.IV & 24.V.1970; ♂ 17123, leg. S. Vanni, 2.I.1986; juv. 19159, young ♂ 19161, 5 ♂♂ 19154, 19155, 19156, 19157 and 19160, ♀ 19158, leg. S. Vanni & R. Cimmaruta, 23.IV.1987;



- 3 juv. 23230, 23231 and 23232 (281-283 NHCL), 5 ♂♂ 23233, 23234, 23235, 23236 and 23237 (284-288 NHCL), 1 ♀ 23238 (289 NHCL), leg. B. Lanza & T. Bianchi, 26.III.1989; 4 juv. 23307, 23308, 23309 and 23310 (3849-3852 NHCL), 2 ♂♂ 23303 and 23304 (3845-3846 NHCL), 2 ♀♀ 23305 and 23306 (3847-3848 NHCL), leg. S. Vanni & B. Lanza, 16.VI.1995; very young spec. 26106 (4451 NHCL), ♂ 26109 (4454 NHCL) now 52716 in MSNG, ♀ 26108 (4453 NHCL), B. Lanza & P. Giorgio Lanza, 22.XI.1997; juv. 26115 (4656 NHCL), 3 ♂♂ 26110, 26111 and 26112 (4651-4653 NHCL), 2 ♀♀ 26113 and 26114 (4654-4655 NHCL), leg. B. Lanza, S. Vanni & G. Nistri, 4.VI.1999;
- cliffs near the confluence, ca. 44°08'02"N-02°24'10"W(Rome), of



Fig. 2 - *Speleomantes ambrosii bianchii* n. ssp.; juv. 26115 MZUF (formerly 4656 NHCL); Tana della Bastiola, 4.VI.1999 (anaesthetized specimen, submerged in water; photo Saulo Bambi).

the Rio della Calda with the Rio Grande, of which Rio della Calda is a left tributary, ca. 1.3 km SSE of Pùlica (church), ca. 300 m (mun. of Fosdinovo; upper valley of the Torrente Pésciola), 4 juv. 26119, 26120, 26121 and 26122 (4660-4663 NHCL), ♂ 26116 (4657 NHCL), ♀ 26117 (4658 NHCL) and ♀ 26118



(4659 NHCL) now 52717 in MSNG, leg. B. Lanza & P. Male-  
notti, 12.X.1998;

- Grotta del Paradisi, c.n. 831 T/MS, 44°08'02"N-02°24'09"W (Rome), 310 m (probably wrongly named Grotta del Ferdì or Grotta del Fredì; with a level indication of 360 m, in the Tuscan Speleological Cadastre; actually the Grotta del Ferdì seems to be a deep well of the same zone, with no c.n., not far from the Grotta del Paradisi); ca. 1.3 km SSE of Pùlica (church) (mun. of Fosdinovo; upper valley of the Torrente Pésciola), ♂ 17124; B. Lanza, S. Vanni & R. Cimmaruta, 23.III.1986; young ♂ 19165, 3 ♂♂ 19162, 19163 and 19164, leg. S. Vanni & R. Cimmaruta, 23.IV.1987;

Table 3 - Teeth number of *Speleomantes ambrosii bianchii* n. ssp. from Pùlica and of *S. ambrosii ambrosii* from La Spezia. pm= premaxillar teeth; av= anterior vomerine teeth; m= maxillary teeth; ma= mandibular teeth; G=G-test.

| Locality | Sex | n  | Teeth | Range | Mean        | cl (99%)    | G               | df | P     |
|----------|-----|----|-------|-------|-------------|-------------|-----------------|----|-------|
| Spezia   | ♂♂  | 12 | pm    | 1-4   | 2.50±1.000  | 1.60-3.40   |                 |    |       |
|          |     |    |       |       |             |             | no significance |    |       |
| Pùlica   | ♂♂  | 11 | pm    | 1-2   | 1.91±0.302  | 1.62-2.20   |                 |    |       |
|          |     |    |       |       |             |             |                 |    |       |
| Spezia   | ♀♀  | 18 | pm    | 2-6   | 4.56±1.042  | 3.84-5.27   |                 |    |       |
|          |     |    |       |       |             |             | no significance |    |       |
| Pùlica   | ♀♀  | 7  | pm    | 3-5   | 3.86±0.690  | 2.89-4.82   |                 |    |       |
|          |     |    |       |       |             |             |                 |    |       |
| Spezia   | ♂♂  | 12 | av    | 4-19  | 16.33±1.614 | 14.89-17.78 |                 |    |       |
|          |     |    |       |       |             |             | no significance |    |       |
| Pùlica   | ♂♂  | 11 | av    | 13-18 | 15.18±1.834 | 13.43-16.93 |                 |    |       |
|          |     |    |       |       |             |             |                 |    |       |
| Spezia   | ♀♀  | 15 | av    | 14-25 | 18.40±2.823 | 16.23-20.57 |                 |    |       |
|          |     |    |       |       |             |             | no significance |    |       |
| Pùlica   | ♀♀  | 7  | av    | 11-17 | 15.00±2.170 | 12.08-17.92 |                 |    |       |
|          |     |    |       |       |             |             |                 |    |       |
| Spezia   | ♂♂  | 12 | m     | 23-30 | 25.83±2.368 | 23.71-27.96 |                 |    |       |
|          |     |    |       |       |             |             | 23.877          | 8  | <0.01 |
| Pùlica   | ♂♂  | 11 | m     | 16-22 | 0.09±1.868  | 18.31-21.88 |                 |    |       |
|          |     |    |       |       |             |             |                 |    |       |
| Spezia   | ♀♀  | 18 | m     | 20-38 | 29.11±4.575 | 25.99-32.24 |                 |    |       |
|          |     |    |       |       |             |             | 16.563          | 13 | <0.05 |
| Pùlica   | ♀♀  | 7  | m     | 18-24 | 21.86±2.478 | 18.38-25.33 |                 |    |       |
|          |     |    |       |       |             |             |                 |    |       |
| Spezia   | ♂♂  | 12 | ma    | 30-42 | 35.17±3.950 | 31.62-38.71 |                 |    |       |
|          |     |    |       |       |             |             | no significance |    |       |
| Pùlica   | ♂♂  | 11 | ma    | 31-37 | 33.09±2.071 | 31.11-35.07 |                 |    |       |
|          |     |    |       |       |             |             |                 |    |       |
| Spezia   | ♀♀  | 18 | ma    | 30-46 | 38.06±4.633 | 34.89-41.22 |                 |    |       |
|          |     |    |       |       |             |             | no significance |    |       |
| Pùlica   | ♀♀  | 7  | ma    | 27-38 | 32.86±4.741 | 26.21-39.50 |                 |    |       |



- left side of the stream Canale Pescioletta, 44°07'00"N-02°23'00"W (Rome), 2 km E of Pùlica and 1 km SW of Marciaso, ca. 580 m (mun. of Fosdinovo), 2 ♂♂ 18520 and 18521, leg. P. Agnelli, B. Campolmi, B. Lanza & P. Giorgio Lanza, 4.IV.1987.

*D e r i v a t i o n o m i n i s* - We are pleased to dedicate this subspecies to Dr. Tancredi Bianchi (Massa, born on 18.I.1924), odontologist, who, alongside with the senior author, collected the first specimens of the new taxon on the 18<sup>th</sup> of October 1942, in the course of a biospeleological campaign on the Apuan Alps.

*D i a g n o s i s* - A *Speleomantes ambrosii* distinguishable with certainty from the whole complex of the nominate form by multilocus electrophoresis only. The two subspecies are indeed genetically completely differentiated at four diagnostic loci: *Pgm-2*, *NADH-dh*, *Sod-1* and *Ada-1*. Nevertheless, *S. ambrosii bianchii* may be usually distinguished from the parapatric *S. ambrosii ambrosii* by its colouration, since it lacks the dorsal pattern of the “*ambrosii*-type” frequently occurring in the animals inhabiting La Spezia and its surroundings (fig. 1; see also LANZA *et alii* 1995, figs 97-99, 100 (right), 101-102); in this pattern type the back is usually monochrome, brown to blackish, aside from the presence of two light, usually ochreous yellow, often more or less discontinuous dorsolateral stripes (fig. 1). Morphological characters usually significantly different in *S. a. ambrosii* and *S. a. bianchii* (see below) are not discriminant, due to the more or less broad overlapping of their independent deviations from the mean; the ratios 10 (body length : mental gland width) and 11 (body length : mental gland length), where only a scarce overlapping occurs, could deserve further research on more specimens.

*D e s c r i p t i o n o f t h e h o l o t y p e* (figs 3-4) - External morphology corresponding to that of the species (LANZA *et alii* 1995: 44-45). Head moderately depressed (head width: head depth = 1.88), its posterior portion feebly convex; snout obtusely truncate (this character feebly or not appreciable in the fig. 3!). Measurements (in mm) and ratios: tl= 107; bl= 61; ag= 31.6; tal= 46; hl= 10.6; lg= 13.4; sl= 4.8; id= 3.7; od= 3.5; icd= 4.9; iod= 3.3; ed= 3.7; mgw= 4.1; mgl= 3.5; se= 2.15; fl= 18.6; hil= 19.1; fol= 8.0; 1= 2.33; 2= 1.93; 3= 3.28; 4= 3.19; 5= 1.03; 6= 5.75; 7= 6.49; 8= 4.55; 9= 7.63; 10= 14.88; 11= 17.43; 12= 1.17; 13= 2.15; 14= 1.13; 15= 2.12; 16= 2.21; 17= 2.86; 18= 3.03; 19= 2.16; 20= 3.21; 21= 2.86; 22= 1.12.





Fig. 3 - *Speleomantes ambrosii bianchii* n. ssp.; left to right: ♂ 26107 MZUF (formerly 4452 NHCL, holotype), juv. 26106 MZUF (4451 NHCL), ♂ 26109 MZUF (4454 NHCL) now 52716 in MSNG, ♀ 26108 MZUF (4453 NHCL); Tana della Bastiola, 11.X.1997 (26107 MZUF) and 22.XI.1997 (dorsal view; anaesthetized specimens, submerged in water; photo Saulo Bambi).

Approximate number of teeth on each side: 2 pm, 16 av, 16 m, 32 ma.

Dorsal parts with very reduced dark ground colour, a rather uniform and thick light brown to ochreous brown marbling, and a hint of strongly discontinuous ochreous yellow dorsolateral stripes; a very irregular black V on the neck. Ventral parts dark bistre, dusted and speckled with off-white dots.

Description of the paratypes (figs 2-4) - Their morphological characters, apart from sex and age differences peculiar to the genus, correspond well to those of the holotype. The specimens of *S. ambrosii ambrosii* from La Spezia and environs



(holotype included) and *S. ambrosii bianchii* from Pùlica-Marciaso and environs (holotype included) show the characters reported in tables 2, 3 and 4.

Table 4 - Number of vertebral elements of *Speleomantes ambrosii bianchii* n. ssp. from Pùlica and of *S. ambrosii ambrosii* from La Spezia. tv = trunk vertebrae, pv = postsacral vertebrae.

| Locality | Sex | n  | Vertebrae | Range | Mean        | cl (99%)    | G               | df | P |
|----------|-----|----|-----------|-------|-------------|-------------|-----------------|----|---|
| Spezia   | ♂♀  | 37 | tv        | 13-14 | 13.32±0.475 | 13.12-13.53 |                 |    |   |
|          |     |    |           |       |             |             | no significance |    |   |
| Pùlica   | ♂♀  | 19 | tv        | 13-14 | 13.21±0.419 | 12.93-13.49 |                 |    |   |
|          |     |    |           |       |             |             |                 |    |   |
| Spezia   | ♂♀  | 33 | pv        | 23-28 | 25.79±1.244 | 25.23-26.35 |                 |    |   |
|          |     |    |           |       |             |             | no significance |    |   |
| Pùlica   | ♂♀  | 18 | pv        | 25-27 | 25.83±0.718 | 25.35-26.32 |                 |    |   |



Fig. 4 - *Speleomantes ambrosii bianchii* n. ssp.; same specimens of fig. 3, ventral view (anaesthetized specimens, submerged in water; photo Saulo Bambi).



The following ratios and the approximate tooth number turned out to be significantly different between ♂♂ and ♀♀ of the same locality: 2 ( $P < 0.01$  Spezia), 3 ( $P < 0.05$  Pùlica), 6 ( $P < 0.02$  Spezia), 14 ( $P < 0.05$  Spezia),  $P < 0.02$  Pùlica), 17 ( $P < 0.02$  Pùlica), 20 ( $P < 0.01$  Pùlica), 21 ( $P < 0.01$  Pùlica), 22 ( $P < 0.02$  Pùlica), pm ( $P < 0.01$  Spezia and Pùlica), m ( $P < 0.05$  Spezia and Pùlica), ma ( $P < 0.05$  Pùlica).



Fig. 5 - *Speleomantes ambrosii bianchii* n. ssp.; left to right: ♀ 26113 MZUF (formerly 4654 NHCL), ♀ 26116 MZUF (4657 NHCL), ♂ 26112 MZUF (4653 NHCL), ♂ 26111 MZUF (4652 NHCL), ♂ 26110 MZUF (4651 NHCL); Tana della Bastiola, 4.VI.1999 (dorsal view; anaesthetized specimens, submerged in water; photo Saulo Bambi).

Dorsal colour pattern rather variable, usually similar to that of the holotype (fig. 3), but sometimes recalling that of some *S. italicus* (Dunn), with ground colour abundant or prevailing and ochreous yellow blotches, or the so called “*gormani*”-type, with the dark ground almost completely hidden by an ochreous yellow to ochreous brown colouration. Ventral parts very variable (fig. 3); ground colour dark brown to dark bistre with a pattern of “*italicus*”-type, “*gormani*”-type or intermediate (according to LANZA *et alii* 1995:



45: “1)“*italicus*”-type: dark ground colour dusted or closely dotted with white or off-white; 2) “*gormanii*”-type: dark ground colour with white or off-white, more or less sparse spots and/or vermiculations; occasionally the white guanophores prevail over the dark ground colour”). Especially worthy of mention the tendentially melanic adult ♂ 26116, blackish and spotless dorsally, dark bistre ventrally with thick whitish dots on the throat and a dozen whitish dots on the belly (fig. 6).



Fig. 6 - *Speleomantes ambrosii bianchii* n. ssp.; left to right: ♂ 26116 MZUF (formerly 4657 NHCL), ♀ 26118 (4659 NHCL) now 52717 in MSNG, ♀ 26117 MZUF (4658 NHCL), juv. 26121 MZUF (4662 NHCL), juv. 26120 MZUF (4661 NHCL), juv. 26122 MZUF (4663 NHCL), juv. 26119 MZUF (4660 NHCL); cliffs near the confluence [ca. 44°08'02"N-02°24'10"W(Rome)] of the Rio della Calda with the Rio Grande, near Pùlica, 10.XII.1998 (ventral view; anaesthetized specimens, submerged in water; photo Saulo Bambi).

For further morphological data on other populations of the species see LANZA *et alii* (1995; tables V, p. 69, and VI, pp. 71-93, local samples signed respectively CS for *S. ambrosii ambrosii* and FO for *S. ambrosii bianchii*; table XXXI, pp. 216-224 for the animals of the hybrid zone between *S. ambrosii bianchii* and *S. italicus*).



## CONCLUSIONS

As already hypothesized by NASCETTI *et alii* (1996: 165), the evident lack of gene exchange between the two subspecies of *S. ambrosii* seems to be related to the extrinsic geographic barriers represented by the Magra River and, probably, by unfavourable ecological conditions in the upper course of the river, as suggested by the presumable absence of the genus *Speleomantes* from Pontremoli's area E of the Magra River.

According to the same authors, "plethodontid salamanders have provided instances of high genetic divergence associated with low morphological differentiation" (see also, among others, WAKE *et alii* 1978), what implies that some taxa may be identified only after genetic analyses. Nonetheless some taxa result to be identifiable also on a morphological basis where sympatry between species or parapatry between subspecies occur. The first possibility may be exemplified by the paradigm case of the North-american *Plethodon dorsalis* Cope and *P. websteri* Highton whose colour pattern differs only in sympatry (HIGHTON 1979); the second one is well illustrated by the case of *Speleomantes ambrosii ambrosii* and *S. ambrosii bianchii*.

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## ABSTRACT

The Authors describe Bianchi's Cave Salamander, *Speleomantes ambrosii bianchii* n. ssp., endemic to the Province of Massa-Carrara (NW Tuscany; central Italy), East of the Magra River.

The new subspecies differs from the nominate one, *S. ambrosii ambrosii*, occurring West of the Magra River in the Province of La Spezia (E Liguria), especially from a genetic point of view.

A research on 33 enzyme loci, analysed by means of multilocus allozyme electrophoresis, showed that the two subspecies are genetically completely differentiated at four diagnostic loci; nevertheless, *S. ambrosii bianchii* may be usually distinguished from the parapatric *S. ambrosii ambrosii* by its dorsal pattern. The lack of gene exchange between the two taxa seems to be related to the extrinsic geographic barriers represented by the Magra River and, probably, by unfavourable ecological conditions in the upper course of the river, as suggested by the likely absence of the genus *Speleomantes* from Pontremoli's area East of the Magra River.



## RIASSUNTO

Il geotritone di Bianchi, *Speleomantes ambrosii bianchii* n. ssp. (Amphibia, Caudata, Plethodontidae)

Gli autori descrivono il geotritone di Bianchi (*Speleomantes ambrosii bianchii*), nuova sottospecie propria delle Alpi Apuane della provincia di Massa-Carrara (Toscana NW), distinguibile dal complesso della sottospecie nominale [*S. ambrosii ambrosii* (Lanza, 1955)] solo dal punto di vista genetico, essendo differenziata a livello di quattro loci diagnostici; essa è tuttavia differenziabile di regola anche a livello morfologico dalle popolazioni parapatriche della sottospecie nominale per il diverso disegno dorsale. L'assenza di flusso genico fra i due taxa sembra da mettere in relazione a barriere estrinseche geografiche, nella fattispecie rappresentate dal Fiume Magra e probabilmente da zone ecologicamente sfavorevoli nel tratto superiore del corso d'acqua.